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Dated 28 October 1999

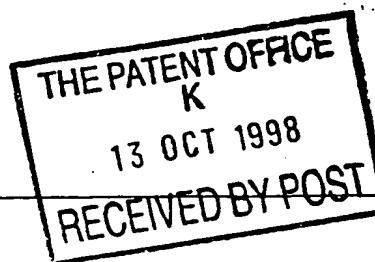
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## Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form.)

The Patent Office  
Cardiff Road  
Newport  
Gwent NP9 1RH

1. Your reference PHM 98-097



2. Patent application number  
(The Patent Office will fill in this part)

13 OCT 1998

9822185.6

3. Full name, address and postcode of the or of each applicant (underline all surnames)

ZENECA Limited  
15 Stanhope Gate  
LONDON W1Y 6LN, Great Britain  
Patents ADP number (if you know it)  
6254007002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

DEVICE

5. Name of your agent (if you have one)  
DENERLEY, Paul Millington

"Address for service" in the United Kingdom to which all correspondence should be sent  
(including the postcode)  
Intellectual Property Department  
ZENECA Pharmaceuticals  
Mereside, Alderley Park  
Macclesfield, Cheshire, SK10 4TG, Great Britain  
Patents ADP number (if you know it) 1030618002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country	Priority application number (if you know it)	Date of filing (day / month / year)
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing (day / month / year)
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

See note (d)

# Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.  
Do not count copies of the same document.

Continuation sheets of this form

Description 8

Claim(s) 2

Abstract

Drawing(s) 3 + 3

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination  
(Patents Form 10/77)

Any other documents  
(please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature

Date 12th Oct 98

Lynda M Slack

12. Name and daytime telephone number of person to contact in the United Kingdom

Mrs Lynda M Slack  
01625 516173

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DEVICE

The invention relates to a microfabricated device for use in measuring the physicochemical or biopharmaceutical properties of compound samples, where the properties measurable using 5 such devices are those which involve partitioning of the compound between two phases, for example, logP, logD, pKa/b, solubility and volatility.

The efficacy of any biologically active compound, for example a pharmaceutical or agrochemical compound, depends on a range of physicochemical and biopharmaceutical 10 attributes of that compound which governs the bioavailability of the compound in the target tissue or organism or at a target molecule. In order to accelerate the rate of discovery of such molecules, there is currently considerable interest in the measurement of such properties very early in the discovery process so that these factors may be used to influence future decisions on which molecules to synthesis as potential drug candidates. However, candidate 15 compounds of potential interest may only be available in relatively small amounts i.e. <1 mg. There is therefore a strong need for methods that can be applied to large numbers of compounds without requiring proportionately more resources and which are capable of dealing with small sample weights. Conventional methods often employ complex separation steps which are time-consuming. Although these can be automated, the serial nature of the 20 analysis means i.e. one sample at a time, still effectively limits the throughput. Automation often requires increased compound amounts which is counter to the thrust of modern synthetic methods such as combinatorial chemistry and multiple parallel synthesis and related technologies which, typically, do not produce large quantities of material.

25 Microfabrication techniques are generally known in the art using tools developed by the semiconductor industry to miniaturise electronics, it is possible to fabricate intricate fluid systems with channel sizes as small as a micron. These devices can be mass-produced inexpensively and are expected to soon be in widespread use for certain simple analytical tests. See, e.g., Ramsey, J.M. et al. (1995), "Microfabricated chemical measurement 30 Systems," Nature Medicine 1:1093-1096; and Harrison, D.J. et al (1993), "Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip," Science 261:895-897.

Miniaturisation of laboratory techniques is not a simple matter of reducing their size. At small scales different effects become important, rendering some processes inefficient and others useless. It is difficult to replicate smaller versions of some devices because of material or process limitations. For these reasons it is necessary to develop new methods for 5 performing common laboratory tasks on the microscale.

Devices made by micromachining planar substrates have been made and used for chemical separation, analysis, and sensing. See, e.g., Manz, A. et al. (1994), "Electroosmotic pumping and electrophoretic separations for miniaturized chemical analysis system," *J. Micromech.*

10 *Microeng.*" 4:257-265.

Microfabricated devices are also known for the extraction of molecules from solution. For example PCT Publication No. WO96/12541 describes a microcontactor device in which two streams flow side-by-side in a microchannel, each stream contains a liquid immiscible with 15 the liquid in the other stream. At such small scales mixing of the flows does not occur and therefore molecules are able to diffuse into the opposite stream prior to the flows being separated again. However, only molecules which are soluble in the opposite stream will be able to cross the phase boundary between the two immiscible liquids. The device is used for the purification of compounds by solvent extraction.

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PCT Publication No. WO97/00442 describes a microcontactor device in which two streams flow side-by-side in a microchannel. Smaller molecules with a much higher diffusion coefficient are able to cross into the opposite stream prior to the flows being separated again. Larger molecules with much larger diffusion coefficients are not allowed sufficient time to 25 diffuse into the opposite stream. The device is used for purification, with the separation of small molecules from large molecules. Also described is that the output of each channel may be analysed to test for the presence of any molecule.

We have found that it is possible to measure in a microfabricated device the physicochemical 30 or biopharmaceutical properties of a single compound if it is allowed sufficient time to partition between two phases or states, where at least one phase or state is then analysed to

provide the information for determining the physical property.

Biopharmaceutical properties include, for example, protein binding, *in vitro* metabolism, passive and active membrane transport. Physicochemical properties include, for example, 5 logP, logD, pKa/b, solubility and volatility.

Each state of a compound may take the form of the compound being, for example, bound or unbound to a biological species or in its ionisation state.

- 10 Each phase of a compound may be, for example, solid, liquid, gaseous or dissolved in aqueous or non-aqueous liquids (preferably organic solvents).

Accordingly, we present as a first feature of the invention a method for the measurement of one or more physicochemical or biopharmaceutical properties of a compound in a

- 15 microfabricated device which method comprises;

(i) providing through an internal surface defining a conduit of the microfabricated device a flow of liquid in which is present the compound;

- 20 (ii) bringing the flow of liquid into contact with an agent within the conduit for a sufficient period for any possible partitioning of the compound from the liquid to the second agent to be substantially completed;

- (iii) measuring the amount of compound or compound derivative present after partitioning in  
25 either the liquid or the second agent, or both.

The compound present in the liquid may be dissolved in the liquid, in the form of solid particles suspended within the liquid or the compound may be in a liquid state.

- 30 In this disclosure, the term liquid means either an aqueous or non-aqueous liquid. In addition the liquid may or may not be a solvate for the compound.

In this disclosure, the term microfabricated includes devices capable of being fabricated on silicon wafers, preferably monocrystalline silicon, readily available to those practising the art of silicon microfabrication and having the feature sizes and geometries producible by such methods as LIGA, thermoplastic micropattern transfer, resin based microcasting, 5 micromolding in capillaries (MIMIC), wet isotropic and anisotropic etching, laser assisted chemical etching (LACE), and reactive ion etching (RIE), or other techniques known within the art of microfabrication. In the case of silicon microfabrication, larger wafers will accommodate a plurality of the devices of this invention in a plurality of configurations. A few standard wafer sizes are 3" (7.5cm), 4"(10cm), 6"(15cm), and 8"(20cm). Application of 10 the principles presented herein using new and emerging microfabrication methods is within the scope and intent of the invention. Microfabricated devices are created through innovative combinations of three essential manufacturing processes: (1) photolithography, the optical process of creating microscopic patterns (2) etching, the process that removes substrate material and (3) deposition, the process whereby materials with a specific function can be 15 coated onto to surface of the substrate.

By partitioned we mean that the compound is allowed to distribute between two phases or states. This partitioning may be allowed to reach equilibrium however it will be appreciated that for certain determinations, for example logP, it may be advantageous to take one or more 20 measurements before equilibrium and deduce the physical property on the basis of extrapolation, or on the basis of a calibration curve. Examples of such partitioning and second agents include: a) partitioning between two different solvents for example for logP measurements b) partitioning between different ionic states in aqueous solution, for example for pKa/b measurements c) partitioning between protein-bound and unbound, for 25 measurements of protein binding d) partitioning between solution phase and a membrane phase for measuring membrane transport e) partitioning between liquid and gas states for measuring volatility.

The agent may be any substance into which the compound may partition. The exact agent 30 will depend upon the physicochemical or biopharmaceutical property being measured and

may be, for example, a miscible or non-miscible liquid, (a solvent, especially organic solvents), a gas, a chemical or a biological reagent.

In this disclosure, the term compound refers to any substance of biological or chemical origin.

5 Compound derivatives are forms of the compound which have been altered in some way by exposure to the liquid or to the agent, for example, solvated, metabolised, ionised, reduced or dissolved.

We present as a further feature of the invention a method for the measurement solubility of a  
10 compound in a microfabricated device which method comprises;

(i) providing into an internal surface defining a conduit of the microfabricated device the compound;

15 (ii) contacting the compound with a liquid within the conduit for a sufficient period for any possible physical partitioning of the compound from solid to dissolved compound to be substantially completed;

(iii) measuring the amount of compound or compound derivative present after partitioning in  
20 the liquid or which remains undissolved, or both.

We present as a further feature of the invention a method for the measurement of one or more physicochemical properties of a compound in a microfabricated device which method comprises;

25

(i) providing through an internal surface defining a conduit of the microfabricated device two liquids;

(ii) the first liquid contains dissolved compound;

30

(ii) the second liquid is immiscible with the first liquid;

- (iii) bringing the two liquids together, preferably in parallel laminar flow, for a sufficient period for any possible partitioning of the compound from the first liquid to the second liquid to be substantially completed;
- 5 iv) detecting or measuring the amount of compound or compound derivative in the first liquid and/or the second liquid.

The term parallel laminar flow means stable flow of the liquid through the conduit, there being no areas of turbulence. Therefore the presence of compound or compound derivative in  
10 the second liquid is entirely due to the ability of the compound to partition between the first liquid and the second liquid, i.e. its physicochemical property.

Typically, parallel laminar flow occurs where there is a combined channel depth of the conduit within which both liquids flow of no more than 100 $\mu$ m. Preferably the  
15 microfabricated conduit which contains the combined flow has a constant width and a smooth internal surface.

In this disclosure, the term liquid means either an aqueous or non-aqueous liquid. In addition the liquid may or may not be a solvate for the compound. The second liquid may differ from  
20 the first liquid in the following ways, the difference, if any, depends upon the physicochemical property to be determined, for example, immiscibility or viscosity.

In the invention the measurement of the compound or compound derivative after partitioning may be achieved either on the device or off the device or a combination of both. Suitable  
25 methods for determination on the devices include potentiometry, conductiometry. Capillary Electrophoresis, Capillary Electrophoresis Chromatography or High Performance Liquid Chromatography with Ultra Violet detection as well as a variety of surface analysis techniques such as Surface Plasmon Resonance, Surface Acoustic Wave. Suitable off-device methods include HPLC with UV or Mass Spectrometry detection. Alternatively a chromatographic  
30 separation could be carried out on the device with spectroscopic detection such as UV or MS being carried out off the device. With parallel laminar flow the two liquids may be separated

downstream after the partition between the two liquids is substantially complete. The separated laminar flow liquid may then be sampled for the presence or absence of compound.

In the case of off-device methods, a suitable interface to the measuring equipment will be required. In the case of mass spectrometry, suitable interfaces have already been described. Further developments in device interfacing may be expected and are hereby incorporated into the device and methods of the invention.

It also may be desirable to measure using on or off-device methods the amount or concentration of compound present in the inlet stream prior to partitioning.

Devices may be conceived where the measurement of more than one property is made simultaneously (e.g. logP measurements using several different solvents) and conversely where the same measurement is made on several different compounds..

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The invention is illustrated by the following non-limiting diagrams.

Figure 1 - shows a schematic diagram of a microfabricated device of the invention where compound in a liquid from a compound sample container 1 is brought into contact with the agent 2 and the amount of compound in each partition is measured at suitable detection points 3.

Figure 2 - shows a schematic diagram of a microfabricated device for measuring the logP of compound where compound in an aqueous liquid from a compound sample container 1 is brought into contact with the agent 2 flowing from an agent container 4, which in this case is an organic solvent. UV light is shone through the microfabricated device and the amount of compound in each partition is measured by MS at suitable detection points 3.

Figure 3 - shows a schematic diagram of a microfabricated device for measuring the protein binding of a compound where compound in an aqueous liquid from a compound sample container 1 is brought into contact with the agent 2, which in this case is a suitable protein

bound to the surface of the device. Measurement of the compound before and after partitioning may be by shining UV light through the device and use of MS at a suitable detection point 3.

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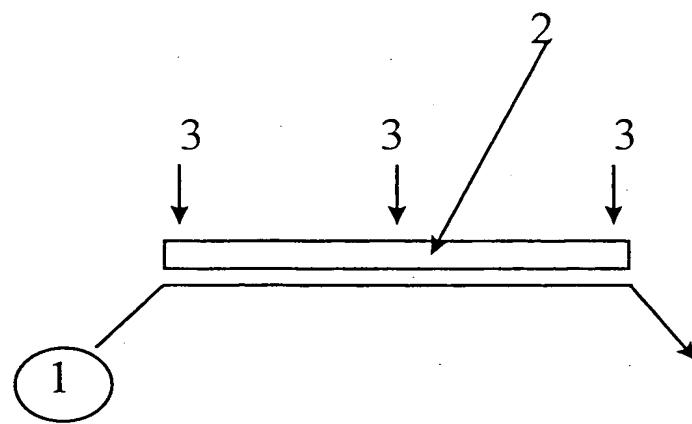


Fig.1

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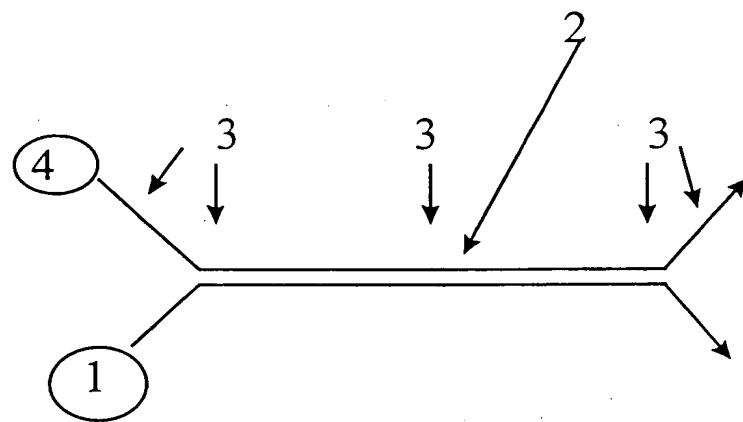


Fig.2

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5 1

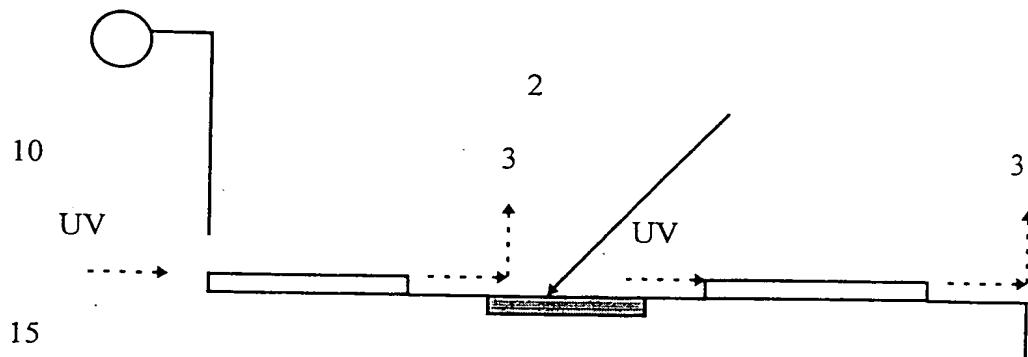


Fig.3

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